

U.S. Patent Application No. 10/088,750  
Amendment dated February 22, 2006  
Reply to Office Action of December 22, 2005

**AMENDMENTS TO THE CLAIMS:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1. (Withdrawn) An RNA higher-order structure having a function for promoting a translation activity which comprises a base sequence selected from the group consisting of:

1) a base sequence expressed by sequences designated in Sequence Nos. 1 to 7 of the sequence list;

2) a base sequence containing the base sequence of 1);

3) a base sequence that has at least about 50% of homology in sequence to the base sequence of 1) and that has a function for promoting a translation activity;

4) a complementary strand of the base sequences of 1) to 3);

5) a base sequence hybridizing with the base sequences of 1) to 4) under stringent conditions; and

6) a base sequence that has been mutated by deletion, substitution, addition, or insertion of one or more base(s) in the base sequences of 1) to 5) and that has a function for promoting a translation activity.

2. (Withdrawn) The RNA higher-order structure according to claim 1 wherein at least PK (pseudoknot) I, II, and III structures are maintained therein.

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3. (Withdrawn) A recombinant vector containing a polynucleotide that comprises at least one base sequence having an RNA higher-order structure having a function for promoting a translation activity which comprises a base sequence selected from the group consisting of;

- 1) a base sequence expressed by sequences designated in Sequence Nos. 1 to 7 of the sequence list;
- 2) a base sequence containing the base sequence of 1);
- 3) a base sequence that has at least about 50% of homology in sequence to the base sequence of 1) and that has a function for promoting a translation activity;
- 4) a complementary strand of the base sequences of 1) to 3);
- 5) a base sequence hybridizing with the base sequences of 1) to 4) under stringent conditions; and
- 6) a base sequence that has been mutated by deletion, substitution, addition, or insertion of one or more base(s) in the base sequences of 1) to 5) and that has a function for promoting a translation activity.

4. (Withdrawn) A transformant that has been transformed with a recombinant vector containing a polynucleotide that is made up of one or more base sequences having a higher order structure having a function for promoting a translation activity, the sequence including a base sequence selected from:

- 1) a base sequence expressed by sequences designated in Sequence Nos. 1 to 7 of the sequence list;
- 2) a base sequence containing the base sequence of 1);

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- 3) a base sequence that has at least about 50% of homology in sequence to the base sequence of 1) and that has a function for promoting a translation activity;
- 4) a complementary strand of the base sequences of 1) to 3);
- 5) a base sequence hybridizing with the base sequences of 1) to 4) under stringent conditions; and
- 6) a base sequence that has been mutated by deletion, substitution, addition, or insertion of one or more base(s) in the base sequences of 1) to 5) and that has a function for promoting a translation activity.

5 - 8. (Canceled)

9. (Previously presented) The method for synthesizing a heterologous protein or a heterologous polypeptide according to claim 20, wherein the synthesis is carried out without using AUG translation initiation codon.

10 - 11. (Canceled)

12. (Withdrawn) The transformant of claim 4 wherein, at least PK (pseudoknot) I, II, and III structures are maintained in the RNA higher-order structure.

13. (Canceled)

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14. (Withdrawn) A method for synthesizing a heterologous protein or a heterologous polypeptide utilizing a transformant which has been transformed with a recombinant vector containing a polynucleotide that is made up of one or more base sequences having a higher-order structure having a function for promoting a translation activity, the sequence including a base sequence selected from:

- 1) a base sequence expressed by sequences designated in Sequence Nos. 1 to 7 of the sequence list;
- 2) a base sequence containing the base sequence of 1);
- 3) a base sequence that has at least about 50% of homology in sequence to the base sequence of 1) and that has a function for promoting a translation activity;
- 4) a complementary strand of the base sequences of 1) to 3);
- 5) a base sequence hybridizing with the base sequences of 1) to 4) under stringent conditions; and
- 6) a base sequence that has been mutated by deletion, substitution, addition, or insertion of one or more base(s) in the base sequences of 1) to 5) and that has a function for promoting a translation activity.

15. (Canceled)

16. (Currently amended) The method for synthesizing a heterologous protein or a heterologous polypeptide according to claim ~~20~~ 22 wherein the synthesis is carried out without using AUG translation initiation codon.

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17. (Previously presented) The method for synthesizing a heterologous protein or a heterologous polypeptide according to claim 27, wherein the synthesis is carried out without using AUG translation initiation codon.

18. (Withdrawn) The recombinant vector according to claim 3, wherein at least PK (pseudoknot) I, II, and III structures are maintained in the RNA higher-order structure.

19. (Withdrawn) The transformant according to claim 4, wherein at least PK (pseudoknot) I, II, and III structures are maintained in the RNA higher-order structure.

20. (Currently amended) A method for synthesizing a heterologous protein or a heterologous polypeptide comprising the steps of

providing a cell-free protein expressing system,

providing a polynucleotide encoding the heterologous protein or heterologous polypeptide and a polynucleotide that promotes translation activity, wherein the polynucleotide that promotes translation activity has an RNA higher-order structure including PK (pseudoknot) I, II, and III structures and is selected from:

1) one of the sequences of SEQ ID NOs: 1-7;

2) a base sequence containing a base sequence of 1); and

3) a base sequence substantially identical to the base sequence of 1) and having the at least PK (pseudoknot) I, II, and III structures or a structure homologous thereto, and

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and wherein the polynucleotide encoding the heterologous protein or heterologous polypeptide is immediately downstream from the PKI structure of the polynucleotide that promotes translation activity, and

translating the polynucleotide encoding the heterologous protein or heterologous polypeptide in the cell-free protein expressing system to form the heterologous protein or heterologous polypeptide, wherein the translating is initiated and/or promoted by the polynucleotide that promotes translation activity.

21. (Canceled)

22. (Previously presented) The method for synthesizing a heterologous protein or heterologous polypeptide according to claim 20, wherein the RNA higher-order structure comprises a base sequence selected from one of the sequences of SEQ ID NOs:1 - 7.

23. (Previously presented) The method for synthesizing a heterologous protein or heterologous polypeptide according to claim 20, wherein the RNA higher-order structure comprises SEQ ID NO:1.

24. (Previously presented) The method for synthesizing a heterologous protein or heterologous polypeptide according to claim 20, wherein the RNA higher-order structure comprises a base sequence selected from one of the sequences of SEQ ID NOs:1 - 7, except that the base sequence contains an alteration in one or more combinations of base pairs that make up

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PKI so that the polynucleotide that promotes translation activity is able to initiate translation activity of a heterologous protein or heterologous peptide without an AUG translation initiation codon.

25. (Previously presented) The method for synthesizing a heterologous protein or heterologous polypeptide according to claim 20, wherein the RNA higher-order structure comprises the base sequence of SEQ ID NO:1, except that positions 187 - 188 of the base sequence of SEQ ID NO:1 are cc instead of uu and positions 159 - 160 are gg instead of aa.

26. (Canceled)

27. (Previously presented) The method for synthesizing a heterologous protein or heterologous polypeptide according to Claim 20 wherein the protein expressing system is a wheat germ extract.

28. (Withdrawn) The method for synthesizing a heterologous protein or heterologous polypeptide according to Claim 20, wherein the protein expressing system is a cell and wherein step of providing a polynucleotide encoding the heterologous protein or heterologous polypeptide and a polynucleotide that promotes translation activity is carried out by transforming the cell with a recombinant vector containing the polynucleotide encoding the heterologous protein or heterologous polypeptide and the polynucleotide that promotes translation activity.

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29. (Withdrawn) The method for synthesizing a heterologous protein or heterologous polypeptide according to Claim 28 wherein the polynucleotide that promotes translation activity is immediately upstream on the recombinant vector from the polynucleotide encoding the heterologous protein or polypeptide.

30. (Currently amended) A method for initiating synthesis of arbitrary heterologous protein or heterologous polypeptide from arbitrary codon which comprises the steps of

providing a cell-free protein expressing system,

providing a polynucleotide encoding the arbitrary heterologous protein or heterologous polypeptide and a polynucleotide that promotes translation activity, wherein the polynucleotide that promotes translation activity is obtained by changing a combination of base pairs that make up PK (pseudoknot) I, II, and III structures in a RNA high-order structure having a function for promoting a translation activity, wherein the polynucleotide encoding the heterologous protein or heterologous polypeptide is immediately downstream from the PKI structure of the polynucleotide that promotes translation activity and wherein the RNA high-order structure has a base sequence selected from:

- 1) ~~one of the sequences of SEQ ID NO: 1 to 7 of the sequence list;~~
- 2) ~~a base sequence containing the base sequence of 1);~~
- 3) ~~a base sequence that has at least about 50% of homology in sequence to the base sequence of 1) and that has a function for promoting a translation activity;~~
- 4) ~~a complementary strand of the base sequences of 1) to 3);~~
- 5) ~~a base sequence hybridizing with the base sequences of 1) to 4) under the~~



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~~following stringent conditions: washing in a solution containing 50% formamide, 5x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA at 42°C, overnight, then washing in 0.1x SSC at about 65°C; and~~

~~6) a base sequence that has been mutated by deletion, substitution, addition, or insertion of one or more base(s) in the base sequences of 1) to 5) and that has a function for promoting a translation activity, and~~

1) one of the sequences of SEQ ID NOs: 1-7;

2) a base sequence containing a base sequence of 1); or

3) a base sequence substantially identical to the base sequence of 1) and having the at least PK (pseudoknot) I, II, and III structures or a structure homologous thereto, and

translating the polynucleotide encoding the arbitrary heterologous protein or heterologous polypeptide from an arbitrary codon in the cell-free protein expressing system to form the heterologous protein or heterologous polypeptide, wherein the translating is initiated and/or promoted by the polynucleotide that promotes translation activity.

31. (Previously presented) The method for initiating the synthesis according to claim 30 wherein, one or more combination(s) of base pairs that make up PK I of the polynucleotide that promotes translation activity is changed, and wherein a base pair maintained in the changed higher-order structure is utilized for said initiating synthesis of said arbitrary heterologous protein or said heterologous polypeptide from said arbitrary codon.